EFFECT OF SITE ON BACTERIAL POPULATIONS IN THE SAPWOOD OF COARSE WOODY DEBRIS

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Abstract—Coarse woody debris (CWD) is an important structural component of southeastern forest ecosystems, yet little is known about its dynamics in these systems. This project identified bacterial populations associated with CWD and their dynamics across landscape ecosystem classification (LEC) units. Bolts of red oak and loblolly pine were placed on plots at each of three hydric, mesic, and xeric sites at the Savannah River Station. After the controls were processed, samples were taken at four intervals over a 16-week period. Samples were ground within an anaerobe chamber using nonselective media. Aerobic and facultative anaerobic bacteria were identified using the Biolog system and the anaerobes were identified using the API 20A system. Major genera isolated were: *Bacillus, Buttiauxella, Cedecea, Enterobacter, Erwinia, Escherichia, Klebsiella, Pantoea, Pseudomonas, Serratia,* and *Xanthomonas.* The mean total isolates were determined by LEC units and sample intervals. Differences occurred between the sample intervals with total isolates of 6.67, 13.33, 10.17, and 9.50 at 3, 6, 10, and 16 weeks, respectively. No significant differences in the numbers of bacteria isolated were found between LEC units.

INTRODUCTION

Coarse woody debris (CWD) may influence a site for several decades in the form of snags, logs, chunks of wood, large branches, or coarse roots. Snags create habitat favorable for cavity nesting birds and animals. Logs in contact with ground may serve as habitat for plants, animals, fungi, and other microorganisms. The degradation process recycles nutrients in the soil and creates a fine-textured material which enhances soil nutrient and energy content, thus creating richer soils for tree growth (Harmon and others 1986, Maser and others 1988, Spies and Cline 1988). Mortality and breakage of living trees add CWD to an ecosystem while fire may remove or transform it (Van Lear 1996). Addition of CWD may occur over time as trees age and die or it may occur sporadically due to disturbances such as hurricanes, tornadoes, and insect and disease epidemics.

At a recent workshop on CWD in southern forests (McMinn and Crossley 1996), emphasis was placed on the need to manage CWD to preserve ecosystem function and health. A conclusion of the workshop was that a lack of knowledge of CWD dynamics is a major limitation to managers. With a better understanding of the CWD loads that could be expected at each stage of forest succession, managers may be able to increase loading during critical periods. Research on CWD dynamics in southern forests is limited to one study (Waldrop 1996) which used a forest-succession model to predict loading. That study suggested that CWD dynamics could be strongly influenced if inputs (limbfall or tree mortality) and outputs (decomposition) of CWD were varied between different types of forest sites. Abbott and Crossley (1982) suggested that decomposition rates vary by site quality.

Decomposition of CWD is a relatively slow process. Factors that control the rate of decay involve temperature, moisture, oxygen, carbon dioxide, and substrate quality (Harmon and others 1986). All of these factors affect the organisms that cause decomposition of CWD. Major microbial organisms that cause decomposition include fungi and bacteria. Insects grind woody components into smaller pieces but do not chemically decompose the wood. Microorganisms degrade cell contents of recently dead woody cells (sugars, starches, proteinaceous materials) and cell wall components (lignin, pectin, hemicellulose, and alpha-cellulose) (Harmon and others 1986). Another population of microorganisms develops to live on the degradation products of these dead microorganisms. Fungi have long been given credit for the majority of the decomposition of wood, but bacteria may also play an important role in the primary breakdown of some woody cell wall components and facilitate the entry of fungi to begin the major part of the decay of CWD.

This study examines the populations of bacteria that occur in CWD placed across three forest site types. These sites were defined using the landscape ecosystem classification (LEC) approach developed by Barnes and others (1982) for forests in Michigan and applied to the South Carolina upper coastal plain by Jones (1991).

This study was performed as part of a long-term, larger research project which examines the decomposition of CWD by site class and species across an environmental gradient at the Savannah River Station (SRS), Aiken, SC. The present study specifically determined the bacterial populations present during the decomposition of sapwood of CWD, by site class and species, within the first 16 weeks following placement of freshly cut wood bolts on the sites.

METHODS

Preparation of Bolts

Boles of loblolly pine (*Pinus taeda* L.) and red oak (*Quercus* spp.) between 20 and 30 centimeters (cm) in

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diameter were cut into half-meter-long bolts. The loblolly pine was collected from the Savannah River Station (Barnwell County, SC) and placed on the study plots within 2 days after cutting; the red oak was collected from the Clemson Experimental Forest (Pickens County, SC) and transported to the Savannah River Station the same day and placed on the study plots the next day.

Location of Study Sites

The USDA Forest Service provided the study plots which were located on the Savannah River Station. They were part of a larger study by McMinn (1997) on the decomposition of CWD by site class and species across a landscape environmental gradient. LEC units were chosen based on their average soil moisture regime and associated understory flora (Jones and others 1984). Three sites of each were selected and included: xeric, mesic, and hydric. The xeric sites were located in pine plantations with little or no undergrowth. The mesic sites were also located in pine plantations; however, there was more undergrowth and debris present on these sites. The hydric sites were located in mixed overstory species stands with dense understories. These latter sites were also located near streams and the soil was very moist at all times. These sites were classified and used in studies on CWD decomposition as affected by microarthropods (Bailey 1994) and fungi (Hare 1992). On each LEC unit, a square plot was established and 11 sample bolts of each species were placed on each plot.

Sample Collection

The sample bolts were collected at 3, 6, 9, and 16 weeks after placement. As a control, a separate bolt was processed immediately after the trees were felled. A randomized system for bolt selection was created for the collection of two bolts of each species from each site during the different sampling periods. The bolts were collected and taken to Clemson University for sampling, breakdown, and analysis. Overall, 162 bolts of wood were sampled.

For bacterial isolations, only the face of each bolt in contact with the ground was sampled. After washing the debarked area with ethanol, a sterile increment borer was used to extract approximately 4 to 5 grams (g) of sapwood. The cores were placed in sterilized, preweighed, screw cap tubes that were continuously filled with nitrogen. The tubes were then weighed to obtain the weight of the core sample. After all cores were collected, they were transferred to an anaerobe chamber.

Culture Preparation

Each core was ground for 5 minutes in 20 milliliters (mL) of anaerobic LPBB (Zeikus and others 1979) using a Sorvall Omni Mixer. With a sterile syringe and needle, 1 mL of this ground material was used to inoculate 9 mL of an anaerobic THAM broth tube (Schink and others 1981). Then, successive transfers were made to dilute the original inoculum. This was done in triplicate. Then, 10 microliters (µL) and 100 µL of the inoculum were used to inoculate anaerobically conditioned THAM agar culture plates. The inoculum was removed from the anaerobic chamber and used to inoculate aerobic dilution tubes of THAM broth and THAM agar plates. After all the samples were processed, the dilution tubes were incubated for 7 days at 30 °C. The anaerobic plates, which were placed in a GasPak jar, and the aerobic plates were incubated for 24 hours (hr) at 30 °C.

At 24 hrs the THAM agar plates were viewed for isolate selection which were then streaked to fresh THAM agar plates in order to obtain pure cultures. At 7 days the dilution tubes were observed for growth and the most probable number (MPN) was calculated. The last positive tube was then streaked for isolation to THAM agar plates.

Identification of Isolates

The aerobic isolates from the MPN tubes were identified using the Biolog Microstation System (Biolog Inc., Hayward, CA, 1993). Biolog is capable of identifying Gram negative, Gram positive, lactic acid bacteria, and yeasts. All isolates were grown and prepared as per Biolog instructions and incubated in microplates for 4 and 24 hours at 30 °C before readings were taken. The anaerobic isolates were identified using the Analytical Profile Index (API) 20A system (BioMerieux Vitek, 1991) which is based on 21 different biochemical reactions. All isolates were grown and prepared as per API instructions and incubated in ampules of API basal medium.

Statistical Analysis

Because several dilutions were missed on the MPN, the bacterial populations were estimated and averaged over LEC unit and sample interval and over tree species and sample interval. No statistical analysis could be performed on these results. An analysis of variance was performed to compare the number of isolates by LEC unit and tree species. Mean separation was by Duncan's Multiple Range Test. Differences were significant at ∂ =0.05.

RESULTS AND DISCUSSION

Aerobic Isolations

All bacteria that were isolated under aerobic conditions and taken from the last positive tube in a serial dilution are summarized in table 1. Overall, 272 organisms were isolated aerobically from the dilution tubes. Of these, 69 were omitted from the identification process for various reasons: 28 did not grow on the medium required for identification, 26 were yeasts, 1 was a fungus, 1 was fastidious, 1 was an actinomycete, and 11 were duplicates. Therefore, 203 isolates were obtained for identification.

Anaerobic Isolations

Only 67 isolates were obtained from the anaerobic dilution tubes. These isolates were also taken from the last positive tube of the dilution series. No strict anaerobes were isolated, only facultative anaerobes similar in genus to the aerobic isolates (table 2). Unlike the aerobic isolates, no yeasts were found. However, *Enterobacter* consisted of 31.7 percent of the anaerobic isolates, with *Erwinia* at 20.6 percent and *Serratia* at 17.5 percent. The only strict anaerobes found in the bolts were those taken from anaerobically conditioned plates of THAM inoculated with the original inoculum of ground core sample in LPBB under

Table 1—Totals by genus of all aerobic isolates and percentages overall of the 257 isolates and the 203 isolates identified by Biolog

Group	Total	Overall	Biolog
		---------Percent ---------	
Enterobacter spp.	47	18.3	23.2
Serratia spp.	21	8.2	10.3
Xanthomonas spp.	16	6.2	7.9
Klebsiella spp.	15	5.8	7.4
Erwinia spp.	15	5.8	7.4
Cedecea spp.	13	5.1	6.4
Pantoea spp.	13	5.1	6.4
Pseudomonas spp.	11	4.3	5.4
Buttiauxella spp.	8	3.1	3.9
Bacillus spp.	7	2.7	3.4
Escherichia spp.	7	2.7	3.4
Curtobacterium spp.	4	1.6	2.0
Other	8	3.1	3.9
Unidentified	18	7.0	8.9
Yeast	26	10.1	n/a
THAM	28	10.9	n/a

Table 2—Totals by genus of the anaerobic isolates and percentages overall of 63 isolates

Group	Total isolates	Percentage
Enterobacter	20	31.7
Erwinia	13	20.6
Serratia	11	17.5
Buttiauxella	3	4.8
Pantoea	3	4.8
Bacillus	2	3.2
Clavibacter	2	3.2
Cardiobacterium	1	1.6
Cedecea	1	1.6
Escherichia	1	1.6
N/I	6	9.5

N/I = Biolog could not identify.

anaerobic conditions (table 3). Only 13 anaerobes were isolated and it could not be determined if these bacteria were present in high numbers in the wood. These bacteria were identified using API 20A and only 4 of the 13 could not be matched to the database.

Differences Between LEC Units

To determine if site type affected the bacterial populations, first the total number of organisms was calculated. From serial dilution tubes, the MPN per gram of core sample was calculated for both the aerobic and anaerobic dilutions. Since many of the dilutions were missed, the estimation of bacterial populations should be higher. Using the MPN/g values, calculated averages by sample period and site type were plotted logarithmically (fig. 1). On all sites, the MPN increased dramatically between week 6 and week 10. However, this pattern and the actual MPN values were nearly identical among LEC units. Analysis of variance showed no differences between the LEC units in total isolates (table 4). The only significant difference occurred between the control and the rest of the LEC units with means of 16.5 for the control, 11.0 for mesic, 9.5 for hydric, and 9.3 for xeric. When comparing the tree species, there was no significant difference between the mean total number of isolates and LEC units. For the xeric and mesic sites the p-values are $p>0.7018$ and $p>0.8312$, respectively.

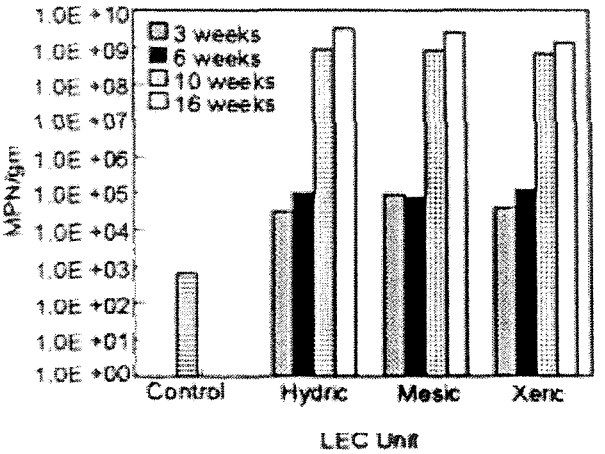

Figure 1—Averages of the most probable number of bacteria per gram of cores sampled from sapwood of red oak and loblolly pine over sample interval and LEC unit.

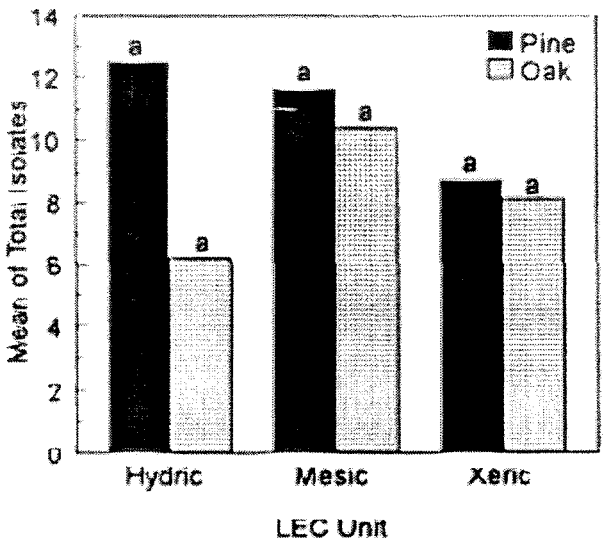

Figure 2—Means of total isolates from the sapwood of red oak and loblolly pine over LEC units. Means within a pair of bars with the same letter are not significantly different at $\partial=0.05$.

Table 3—Bacteria isolated anaerobically from plated samples of red oak and loblolly pine using API 20

Isolate number	Tree number	Tree species	Sample group	Site type	Bacterial organism
AA1	O-2	Oak	0	Control	*Bacteroides oralis*
AA2	O-4	Oak	0	Control	*Bacteroides oralis*
AA3	814B	Pine	1	Mesic 2	*Bifidobacillus adolescentis*
AA4	815B	Pine	1	Mesic 2	N/I
AA5	833	Pine	1	Mesic 3	*Bifidobacillus adolescentis*
AA6	975	Oak	2	Hydric 1	*Streptococcus intermedius*
AA7	867	Pine	3	Hydric 1	N/I
AA8	956A	Oak	3	Xeric 3	*Bacteroides oralis*
AA9	873	Pine	4	Hydric 1	*Clostridium beijerinckii*
AA10	895	Pine	4	Hydric 3	N/I
AA11	898	Pine	4	Hydric 3	*Actinomyces israelii*
AA12	813	Pine	4	Mesic 2	N/I
AA13	825	Pine	4	Mesic 3	*Clostridium beijerinckii*

N/I = API 20A could not identify.

Table 4—Mean total number of isolates by LEC unit

LEC unit	Number	Mean
Control	2	16.500 a
Mesic	8	11.000 b
Hydric	8	9.500 b
Xeric	8	9.250 b

Note: Means with the same letter are not significantly different at ∂=0.05.

Over the hydric sites the p-value is p>0.0728, indicating that there is, likewise, not a difference. However, when comparing the mean values over LEC unit the oak is nearly double that of the pine (fig. 2).

If the isolates are grouped by genus, *Erwinia* and *Xanthomonas* are prevalent in the controls (fig. 3). However, *Enterobacter* was the most abundant in all the LEC units, with the largest number isolated from the mesic site. *Serratia* was also prevalent throughout all LEC units, again with most isolates found on mesic sites. This same trend also pertained to the yeasts that were isolated.

CONCLUSIONS

Previous studies suggested that decomposition varies by site quality, possibly due to different populations or numbers of microorganisms (Abbott and Crossley 1982, Bailey 1994, Hare 1994). In this study, however, bacterial populations were not found to vary, suggesting that they play similar roles in CWD decomposition on hydric, mesic, and xeric LEC units. These results may suggest that bacteria play a minor role in CWD decomposition or that they are highly adaptable to different forested environments. This study identified a large number of facultative anaerobic bacteria from the sapwood of loblolly pine and red oak and created an extensive database of bacteria that inhabit decaying wood of these two tree species.

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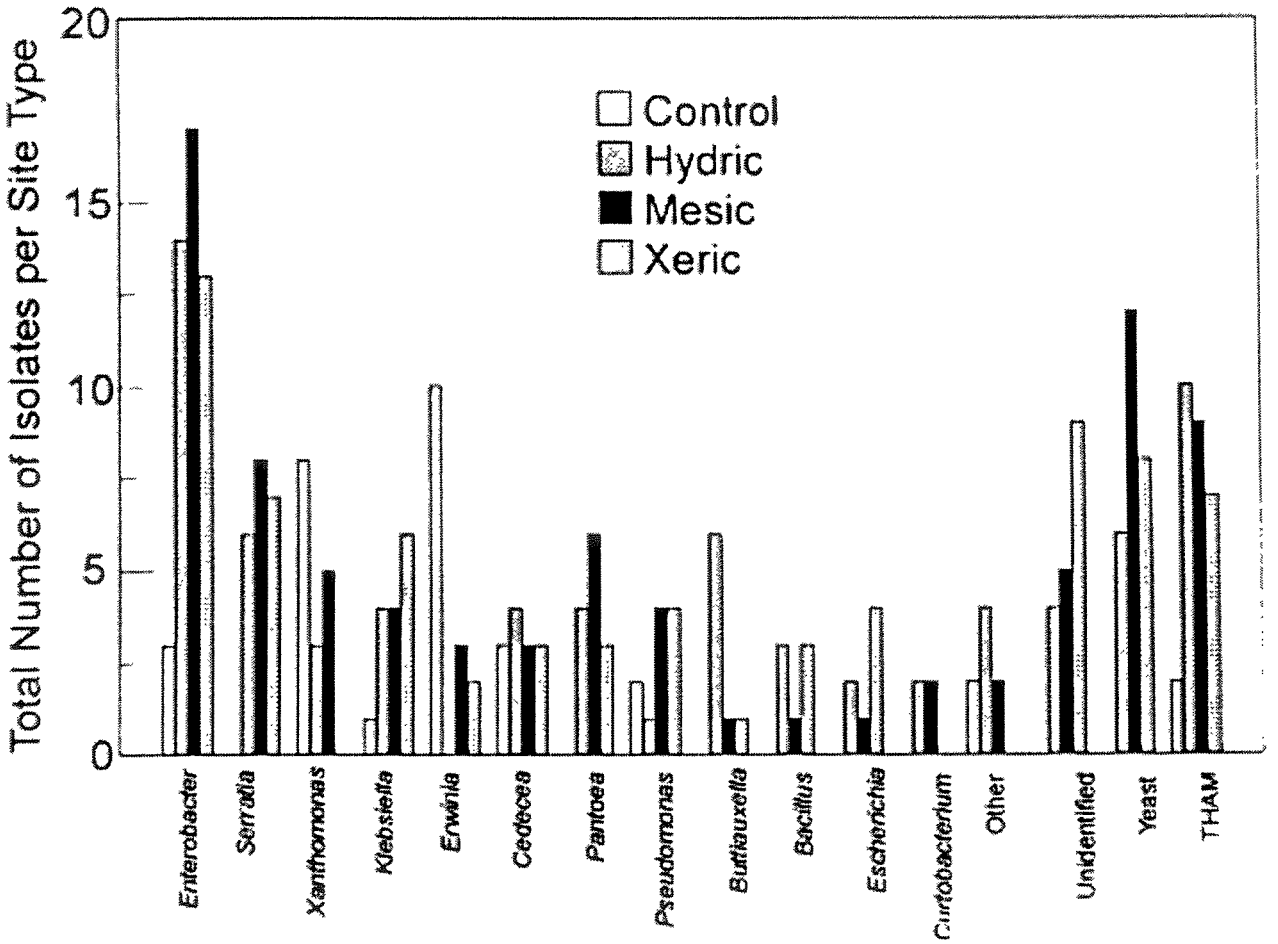

Figure 3—Total number of isolates per LEC unit by genus from the sapwood of red oak and loblolly pine over 16 weeks.

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